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STRUCTURE AND OVARIAN INCUBATION OF GAMBUSIA PATRUELI, A TOP-MINNOW.¹

BY JOHN A. RYDER.

SINCE we have taken up our temporary residence at Cherry-stone we have found this interesting genus of cyprinodonts in great abundance in fresh and brackish water streams, also in a fresh water pond in the vicinity, a few miles south of where our station is located. In the latter situation three forms have been collected all of which are in breeding condition—we will not say spawning condition, as they do not, as do most other fishes, commit their ova to the care of the element in which they live, but carry them about in the ovary, where they are impregnated and where they develop in a very remarkable manner.

Of the manner of impregnation we know little or nothing, except the evidence furnished by the conformation of the external genitalia of the two sexes. In the adult male, which measures one and one-eighth of an inch in length, the anal fin is strangely modified into an intromittent organ for the conveyance of the milt into the ovary of the female; a tubular organ appears to be formed by the three foremost anal rays, but one which is greatly prolonged and united by a membrane. At the apex these rays are somewhat curved toward each other, and thus form a blunt point, but the foremost one of the three rays is armed for its whole length with ridges at its base and with sharp recurved hooks at its tip, the other two at their tips similarly with hooks, and between their tips are two small fenestra or openings which possibly communicate directly with the sperm duct from the testes. The basal elements of the fin are aggregated into a cylindrical columnar truncated bony mass, which is prolonged upward into the cavity of the air-bladder for the distance of nearly the eighth of an inch; from it a series of fibrous bands pass to the dorsal and posterior wall of the air-bladder to be inserted in the median line. Whether this bony column serves to steady the fin in the act of copulation, or whether it serves to give passage to the sperm duct, is an unsettled question with the writer. The modified anal fin of the male measures a third of an inch in length. Other peculiarities of the male are noticeable—for instance, as the more abbreviated air-bladder or space which also occupies a more oblique

¹ From the *Forest and Stream*, New York, Aug. 18, 1881, with notes and corrections.

position than in the female. The most remarkable difference presented by the male as compared with the female, however, is his inconsiderable weight, which is only 160 milligrammes, while that of the gravid female is 1030 milligrammes, or nearly six and one-half times the weight of the male.

The female, as already stated, is larger than the male, and measures one inch and three-fourths in length. The liver lies for the most part on the left side. The intestine makes one turn upon itself in the fore part of the body cavity and passes back along the floor of the abdomen to the vent. The air-bladder occupies two-fifths of the abdominal cavity, and at its posterior end the wolffian duct traverses it vertically, to be enlarged near its outlet into a fusiform urinary bladder of very much the same form as in many embryo fishes. The ovary is a simple, unpaired organ which lies somewhat to the right and extends from the anterior portion of the body cavity to its hinder end, and serves to fill up its lower moiety when fully developed. The ova, when full grown, are each enveloped in a sac or follicle supplied with blood from a median vascular trunk which divides and subdivides as it traverses the ovary lengthwise in a manner similar to that of the stem to which grapes in the bunch are attached. In this way it happens that each egg or ovum has its own independent supply of blood from the general vascular system of the mother, from which the material for the growth and maturation of the egg is derived, and which afterward becomes specialized into a contrivance by which the life of the developing embryo is maintained while undergoing development in their respective follicles in the ovary or egg-bag. The ova develop along the course of the main vessel and its branches, as may be learned upon examining a hardened specimen, where the very immature ovarian eggs are seen to be involved in a mesh-work of connective fibrous tissue, which serves not only to strengthen the vessels but also afterward enters into the structure of the walls of the ovarian sacs or follicles externally.

The very immature eggs measure from less than a hundredth of an inch up to a fiftieth, and on up to a twelfth of an inch, when they may be said to be mature. They develop along a nearly median rachis or stalk which extends backward and slightly downward, and which gets its blood supply very far forward from the dorsal aorta. The ova, after developing a little way, are each inclosed in a follicle, the Græfian follicle, ovisac, ovarian capsule,

membrana granulosa of Von Baer, or *membrana cellulosa* of Coste. As the egg is matured there is a space developed about it which is said to result from the breaking up of the granular layer of cells covering it. This space is filled with fluid, and in this liquid, which increases in quantity as development proceeds, the embryo top-minnow is constantly bathed. *There is no trace whatever in the egg of this fish of an independent egg membrane*, as is the case with all known forms which spawn directly into the water, and which is usually, if not in all cases, perforated by one or more micropylar openings or pores for the entrance of the spermatozoon. This fact raises the question whether the egg membrane or *zona radiata* usually present in the ova of water-spawning fishes is not entirely absent in all the viviparous species. Whether Rathke has recorded anything on this point in his account of the development of *Zoarces*, the viviparous blenny, I am not able to say at present, as I do not have access to his memoir.¹ Suffice it to say, however, that with very cautious preparation, staining and dissection of the follicles inclosing the ova of *Gambusia*, I have completely failed to discover what I could regard as an egg membrane, although personally familiar with the appearance of the coverings of the ova of more than twenty species, embracing fifteen or more families. The *zona radiata* or covering of the egg in other bony fishes is said to be secreted from the cells lining the follicles and is composed of a gelatinoid substance, and it is often perforated all over by a vast number of extremely fine tubules, called pore canals by their discoverer, Johannes Mueller. No such structure existing as a covering for the egg of *Gambusia*, we are in a position to ask the question why such an unique condition of affairs should exist in this case? The answer, it would appear to us, is not far to seek. In the case of eggs which ordinarily hatch in water it is necessary that they should be supplied with a covering more or less firm and capable of protecting the contained embryo, which in the case of the top-minnow is not needed, because the embryo is developed so as to be quite competent to take care of itself as a very well organized little fish

¹ Rathke's description accords pretty closely with my account of the egg follicles of *Gambusia* given farther on. The narrow, elongate stigma, devoid of vessels, on the follicle, spoken of on page 4 of his memoir on *Zoarces*, probably corresponds to what I have called the *follicular foramen*. He has described a vascular network in the follicle, a stalk joining it to the vascular rachis and a space around the yolk much as in *Gambusia*.

when it leaves the body of its parent. Nature will not waste her powers in an effort to make useless clothes for such of her children as do not need them; on the contrary, she is constantly utilizing structures economically, and often so as to serve more than one purpose. This is the apparent answer to the query with which we started.

The follicles or sacs containing the ova are built up internally of flat, polygonal cells of pavement epithelium, and externally of a network of multipolar, fibrous, connective tissue cells and minute capillary blood vessels, with cellular walls, which radiate in all directions over the follicle from the point where the main arterial vessel joins the follicle, and which, together with its accompanying veins and investment of fibrous tissue, constitutes the stalk by which the follicle and its contained naked ovum is suspended to the main arterial trunk and vein. The capillary system ends in a larger venous trunk, which also follows the course of the main median arterial trunk back to the heart by way of the Cuvierian ducts. The very intricate mesh-work of fine vessels which covers the follicle supplies the developing fish with fresh oxygen, and also serves to carry off the carbonic dioxide in much the same way as the placenta or after-birth performs a similar duty for the young mammal developing in the uterus of its parent. There is this great difference, however, between the fish and the mammal. In the former there is no uterus; the development takes place in the follicle in which the eggs have grown and matured; there is no true placenta, but respiration is effected by a follicular mesh-work of blood vessels, and the interchange of oxygen and carbonic dioxide gases takes place through the intermediation at first of the fluid by which the embryo is surrounded in its follicle, and later when blood vessels and gills have developed in the embryo they, too, become accessories to aid in the oxygenation of its blood. In the mammal there is a uterus; the egg must leave its ovarian follicle; be conveyed to the uterine cavity before a perfectly normal development can begin; there is a fully developed richly vascular placenta joined to the foetus, the villi or vascular loops of which are insinuated between those developed on the maternal surface of the uterine cavity. In both fish and mammal, however, this general likeness remains; that there is no immediate vascular connection between mother and embryo. In both the respiration of the embryo is effected by the transpiration of

gases through the intermediation of membranes and fluids, oxygen being constantly supplied and carbonic dioxide carried off by means of a specialized portion of the blood system of the maternal organism.

There is still another difference which distinguishes the developing fish from the mammal, which has not been noticed. The body of the former is built up by a gradual transformation or conversion of the substance of the yelk into the various structures which make up its organization. In other words, the young fish obtains no nutrition from its parent; there is merely an incorporation of the stored protoplasm of the yelk sack. In the mammal, on the other hand, the embryo receives nourishment through the placental structures, the largest proportion of the embryo is built up from the protoplasm supplied from the blood system of the parent. Judging from the large size of the young of some viviparous fishes, such as in *Embiotoca*, it is possible that there may be some exceptions to the rule indicated above.

Besides the very intricate network of capillary vessels which covers the follicles of the ovary of *Gambusia*, a large opening of a circular or oval form makes its appearance in the wall of each one at or near the point of attachment of the vascular stalk by which they are supported. This opening appears to increase in size as the young fish develops; whether it is present during the earliest stages of the intrafollicular development of the embryo I do not know, as I did not have an opportunity to see those phases. A branch from the main nutritive vessel frequently lies near the margin of the opening, curving around it. Whether this opening serves the same purpose as the micropyle of ova provided with a membrane, would appear very probable, as it is difficult to see in what other manner the milt, which is probably introduced into the ovarian cavity by the male, could reach the ovum through the wall of its follicle. The opening into the follicle may be named the *follicular foramen*. Through it the cavity in which the embryo lies is brought into direct communication with the general ovarian space.

We found ourselves unable to determine the species of the form, the structure of which is described above; none of those described in Jordan's *Manual* appear to agree with our species. It may be, as some of us have surmised, that the isolation of the form on the eastern peninsula of Virginia, for a great length of

time, may have served to develop specific characters, and that it is undescribed. We leave the determination of the species to the systematic ichthyologists.¹

Thus far our account has dealt only with the structure of the adults and the peculiar contrivances by means of which reproduction is effected; we will now take up the discussion of the egg and the embryo.

The globular vitellus measures about a line in diameter including the embryonic or germinal portion. The germinal protoplasm probably occupies a peripheral position covering the nutritive or vitelline portion of the egg as a continuous envelope with strands of germinal matter running from it through and among the corpuscles of the vitellus. This peripheral germinal layer, when the egg is ready to be fertilized, migrates toward one pole and assumes a biscuit shape. This is essentially the history of the formation of the germinal disk of the Teleostean egg as worked out independently by Coste, Kupffer and the writer. Little of a trustworthy character is known of the history of the germinative vesicle and spot, which bear the same relation to the egg as the nucleus and nucleolus do to the substance of the cell of the ordinary type. When cleavage of the germinal disk has begun, *it is the first positive evidence that impregnation has been successful*. The disk then begins to spread over the vitellus or yelk and soon acquires the form of a watch glass, with its concave side lying next to the surface of the yelk. Coincident with the lateral expansion of the germinal disk, a thickening appears at one point in its margin which is the first sign of the appearance of the embryo fish. With its still further expansion, the embryo is developed more from the margin of the disk toward its center; in this way it happens that the axis of the embryo lies in one of the radii of the disk; its head toward the center, its tail at the margin.

But before the embryo is fairly formed, a space appears under the disk, limited by the thickened rim of the latter, and the embryo at one side. This space, the segmentation cavity,² *is filled*

¹Our original reference of this fish to *Zygonectes* has proved to be erroneous, the species proves to be *Gambusia patruelis* of Baird and Girard. Its discovery north of the mouth of the Chesapeake marks the northernmost limit of its occurrence yet known, most of the members of the genus being sub-tropical and West Indian.

²This cavity is the exact homologue of that in the batrachian ovum. In the fish and bird it is somewhat modified, and no doubt serves to enable the blastoderm to spread over the yelk as segmentation proceeds.

with fluid and grows with the growth of the germinal disk, as the latter becomes converted into the blastoderm, and does not disappear until some time after the embryo has left the egg as a young fish ; and then it often remains as a space around the yelk sac for as long as a vestige of the latter remains, as may be seen in the young of *Cybius*, *Parephippus*, *Gadus*, *Elacate* and *Syngnathus*. In regard to this point, I hold views entirely different from any other observers, but inasmuch as the writer has had opportunities for the study of the development of a greater number of species, representing a greater number of families, than any previous investigator, and because the observations are based on material studied without the use of hardening re-agents which either deform or obliterate the segmentation cavity, and also because it was found to be present in all of the forms which were sufficiently well studied, it is believed that it will be found in the developing ova of most or all Teleostean fishes. Should this prove to be the fact, the Teleostean egg will be as distinctly defined in respect to the sum of the developmental characters which it presents, from the developing ova of other vertebrates, as the adult Teleost is from the remaining classes of the sub-kingdom to which it belongs. The floor of the cavity appears to be formed by the hypoblast or innermost embryonic layer, while its roof is formed by the epiblast or outermost skin layer. Gradually this blastoderm, which has been derived by cleavage from the germinal disk, grows over the yelk, no part of its epiblast layer being in direct contact with the hypoblast below on account of the presence of the intervening film of fluid, except at its rim. The embryo is also found to be in fixed contact with the yelk. The blastoderm grows at about an equal rate all around its margin ; the point where the edges of the blastoderm finally close is almost directly opposite the site where the germinal disk first appeared ; the closure at last occurs just behind the tail of the embryo where a little crater-like elevation marks the point at which it disappears. The embryo now lies along a meridian of the blastoderm ; its head at the original germinal pole, its tail at the other. The growth of the blastoderm over the yelk is greatly facilitated by the film of fluid contained in the segmentation cavity, over which it can glide as it grows without friction. This view seems to me to be the most rational yet proposed in explanation of the method by which the blastoderm grows laterally in all directions

down over the yelk. In some cases the yelk sac is frequently much absorbed before the outer epiblastic sac begins to collapse. This is the case with *Cybum* after it leaves the egg, and proves very conclusively that the outer sac is entirely free, laterally and ventrally, from the inner one containing the yelk.

There are two principal methods by which the yelk is absorbed; the one where a more or less extensive net-work of vessels is developed over the surface of the yelk, and through which all, or nearly all, of the blood passes to reach the venous end of the heart; in many cases no such net-work is ever developed, as for instance, in the shad, mackerel cod and bonito. To the former class the young top-minnow belongs. Its yelk is orange-colored and imbedded in it superficially are a great number of refringent oil globules of small size. There appears to be a sinus beneath the head, continuous with the segmentation cavity in which the heart is developed. The body of the young fish lies in a groove or furrow on the surface of the yelk. This is the youngest state in which I have seen *Gambusia*, and explains why I have given the preceding general account of the development of a young fish. The somites or segments of muscle plates had been developed for some time. The heart, brain, intestine and organs of sense were defined.

The next important stage observed, was when the yelk sac was in great part absorbed and the fish nearly ready to hatch, or more properly to leave its follicle and the body of its parent. The extraordinary acceleration of development noted in almost every detail of structure, was such as I had never witnessed in any other species of young fish. The bones of the skull, although still cartilaginous, were advanced to a condition not seen in the shad until it has been hatched for three weeks or more. There were intermaxillary elements with teeth; pharyngeal patches of teeth; the brain was pretty well roofed over by the cartilaginous cranium; the branchiosteges were developed in cartilage; the opercles completely covered and concealed the gills, the opercular elements being differentiated; the gills already bore branchial leaflets; the neural and haemal arches of the vertebræ were being developed in cartilage; scales covered the sides and back and were developing in pockets of the dermal epithelium; in fine, all the fins were already developed except the ventrals with the same number of rays as in the adult, and yet the yelk sac was not nearly

absorbed. I have never seen in any fish embryos of the same age, an instance where scales were developed or where the fins had approximated their adult condition so nearly as in this case. The only instance known to me at this writing where a continuous dorsal and ventral median fin-fold is never developed, is in the case of *Syngnathus*, where the caudal rays are developed before the dorsal ones. Whether the unpaired fins of *Gambusia* are or are not derived from such a fold would be an interesting observation. A marked acceleration is also noticeable in the development of the brain, a study of which, by means of sections, as compared with that of the adult, has furnished me with some valuable clues in following up the development of Teleostean brains in general.

To sum up, this fish begins an independent career as far developed as when the shad, cod, mackerel, bonito and many other fishes are from three to six weeks old. By so much it has the advantage over those types in the struggle for existence in that it is ready to feed, to pursue its prey discriminately, as soon as it is born, while the other forms alluded to are comparatively helpless until some time after they have absorbed their yelk sacs, although most of them by that time have acquired mandibular, maxillary or pharyngeal teeth or both. The Fish Commission authorities need never be uneasy about the fate of the top-minnows; they will take care of themselves; their species is sure of survival. But our study, it would seem to the writer, has not been in vain, because, even though the fish is too small to be of any practical value, it has taught us that where nature has so effectually provided for the protection of the young fish, she does not require one adult to produce as many embryos. In *Gambusia* twenty-five to thirty young is perhaps the limit of production for a single female; in *Apeltes*, or the four-spined stickleback, the male of which is provided, according to my observations, with a spinning apparatus with which he fabricates a nest in which the young are hatched and taken care of, the number of eggs is from fifteen to twenty. Contrasting these small numbers with 100,000 to 3,000,000, the number of ova easily matured in a single season by a single female of many anadromous and marine species which have heavy, adhesive or floating eggs, it would appear that the quantity of germs produced by different species of fishes is in some way proportioned to their chances of survi-

val. Otherwise we are at a loss to explain the enormous fertility of many marine forms; the astounding fertility of the oyster and clam are other instances illustrating this principle, where ova are matured by the tens of millions, but where barely one out of a million survives so as to attain adult age.

Certain adaptations of structure are also plainly noticeable on a comparative study of fish ova. Thus the egg membrane of floating eggs is extremely thin, thinner than that of heavy or adhesive eggs, while the thickest membranes are those provided with external filamentous appendages. The most thinly clad hatch out soonest. May it not be that the thinness of the envelope of the egg has some relation to the rapidity with which the oxygenation of the egg is effected, and consequently with the rapidity of tissue and embryonic changes? And, finally, who would undertake to say that all of these modifications of the embryonic envelope are not such as could be developed by natural selection so as to favor the survival of the greatest number of embryos?

Many other general views of a similar character might be drawn from the material in my possession, but I fear that there has been already too much detail entered into for this note to be of interest to the general reader.

Before closing I wish to state that it is the oviduct of the female in some cyprinodonts that is prolonged into a tube at the anterior edge of the anal fin. This difference, as compared with *Gambusia*, would be useful as a generic character, as suggested by Colonel Marshall McDonald, to whose unselfish, helpful interest I am deeply indebted for assistance in manifold ways, while the investigation of the material was in progress, upon which the foregoing account is based.¹

¹ The only memoir which I have been able to find bearing on the development of a cyprinodont is that by M. Duvernoy, *Sur le développement de la Pécilia surinamensis*, *Ann. Sci. Nat.*, 3 Ser., I. 1844. His account has however been based upon alcoholic material, but shows the remarkable acceleration of development of the embryos the same as in *Gambusia*. The number of embryos, their arrangement in the ovary, and the position of the ovary itself appear also to be similar.